Endothelial Adherens Junctions

Peter Petzelbauer, Thomas Halama, and Marion Gröger

Department of Dermatology, Division of General Dermatology, University of Vienna Medical School, Vienna, Austria

The principle of the molecular organization of adherens junctions follows a uniform pattern, which is found in epithelial, muscular, neuroneal as well as in endothelial cells and is highly conserved among species. Transmembrane molecules of the cadherin family link to catenins, which anchor the adhesion plaque to the cytoskeleton. The kind of cadherin used in adherens junctions is cell-type specific, vascular endothelial (VE)-cadherin is specific for endothelial cells. The assembly and disassembly of the cadherin/catenin complex is dynamic and regulated by growth factors. The functional status of adherens junctions controls endothelial cell-to-cell adhesion, cell scattering, vessel morphogenesis and has intracellular signaling properties, thereby playing an important role in vasculogenesis and angiogenesis.
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MOLECULES INVOLVED IN THE FORMATION OF VASCULAR ADHERENS JUNCTIONS

Cell adhesion is crucial for the assembly of cells into three dimensional tissues. One of the most important and ubiquitous types of adhesive interaction between cells is mediated by cadherins. Cadherins are transmembrane, Ca^{2+}-dependent receptors, which after dimerization and clustering form antiparallel homotypic adhesive complexes with neighboring cells (Steinberg and McNutt, 1999). Many studies found a correlation between cadherin expression or function and the formation of tissue boundaries, tissue rearrangement, cell migration, cell differentiation, and metastasis (Tepass, 1999). Many tissue-specific cadherins have been identified, including epithelial (E)-cadherin, neuroneal (N)-cadherin, placental (P)-cadherin, vascular endothelial (VE)-cadherin, and others. The disturbed expression or function of such individual cadherins results in abnormal development of the respective organs (Larue *et al*, 1994; Radice *et al*, 1997; Vittet *et al*, 1997; Carmeliet *et al*, 1999).

Although the ectodomain of cadherins is sufficient to mediate cell aggregation, it is the cytodomain of cadherins, which contains several functional elements, that allows cadherin-dependent cell-to-cell adhesion to be regulated. The membrane proximal cytoplasmic region of cadherins binds to the catenin $p120^{ctn}$ (formerly called $p120^{CAS}$). Depending on the state of $p120^{ctn}$, this molecule can enhance or inhibit cadherin-mediated cell adhesion (Aono *et al*, 1999). Adjacent and distal to the $p120^{ctn}$ binding site, the cytodomain of cadherins interacts with β-catenin or γ-catenin (also called plakoglobin). These two catenins bind to α-catenin, which is an actin-binding and actin-bundling molecule linking the adhesive cadherin/catenin complex to the F-actin-based cytoskeleton (Provost and Rimm, 1999). Other binding partners of α-catenin are vinculin, α-actinin, ZO-1, and possibly spectrin.

The protein complex between cadherins, catenins, and F-actin shapes the so-called adherens junction. The principle architecture of such junctions is preserved among species and comparable even between invertebrates and vertebrates. The type of cadherin found in the respective species and tissue is specific and determines tissue-specific morphogenesis and functions. The unique cadherin found in adherens junctions of human endothelial cells is vascular endothelial (VE)-cadherin (Breviario *et al*, 1995; Dejana, 1997). Endothelial cells express two other cadherins, vascular endothelial (VE)-cadherin-2 and N-cadherin (Salomon *et al*, 1992; Teloç *et al*, 1998), but these two cadherins do not participate in endothelial adherens junction formation and do not anchor to the cytoskeleton. VE-cadherin merges into adherens junctions as early as 2 h after seeding of endothelial cells onto tissue culture plastic. Then VE-cadherin forms multiple lateral patches that develop into an extensive belt-like structure over a period of 24 h (**Fig 1**). Interestingly, light and electron microscopic studies in endothelial cells have shown that also another cell-to-cell adhesion molecule called platelet endothelial cell adhesion molecule-1 (PECAM-1; CD31) becomes progressively associated with adherens junctions (Leach *et al*, 1993; Ayalon *et al*, 1994). Moreover, like VE-cadherin, PECAM-1 also physically interacts with β-catenin and thus participates in shaping the endothelial adherens junction (Ilan *et al*, 1999; Matsumura *et al*, 1997).[1]

ADHERENS JUNCTION FUNCTION

In angiogenesis The importance of VE-cadherin for vessel formation is emphasized by the fact that a VE-cadherin knockout mouse is unable to form vascular structures and dies around day 10 during embryonic development (Carmeliet *et al*, 1999). Using endothelium in cell culture it was possible to show that VE-

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Reprint requests to: Dr. Peter Petzelbauer, Department of Dermatology, University of Vienna Medical School, Waehringer Guertel 18–20, A-1090 Vienna, Austria. Email: Peter.Petzelbauer@akh-wien.ac.at

Abbreviations: FGF, basic fibroblast growth factor; PECAM-1, platelet endothelial cell adhesion molecule 1; VE-cadherin, vascular endothelial cell cadherin.

[1]Halama T, Groger M, Pillinger M, *et al*: PECAM-1 and VE-cadherin cooperatively regulate fibroblast growth factor-induced modulations of adherens junction functions. *J Invest Dermatol*, submitted.

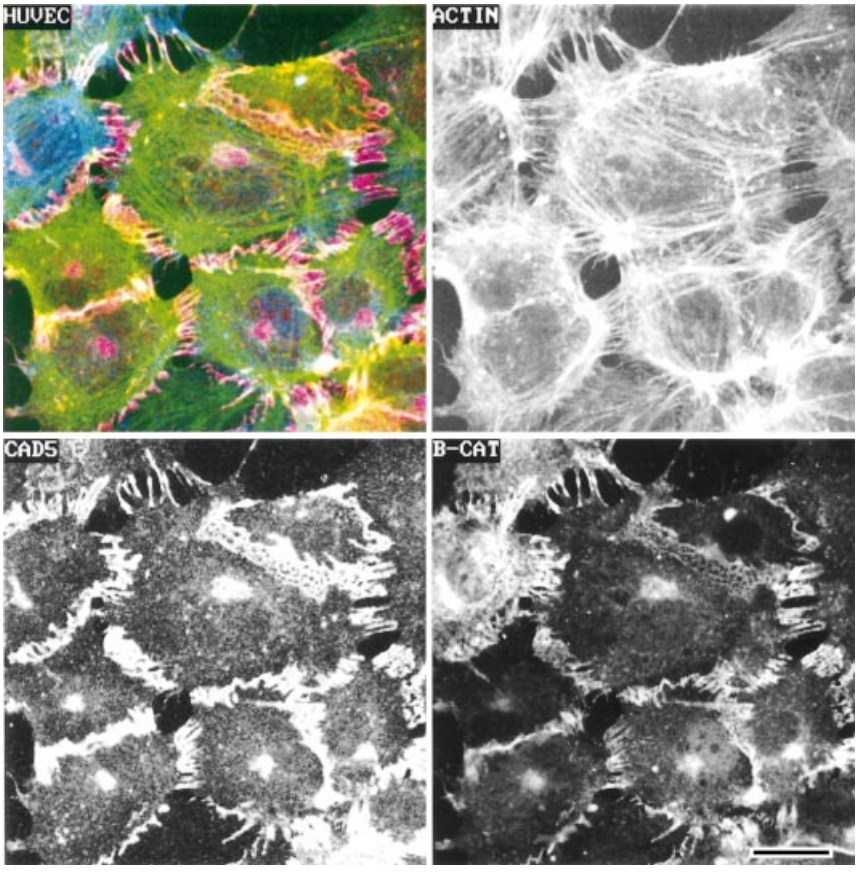

Figure 1. Three color immunofluorescent staining of endothelial cells grown as monolayers on tissue culture plastic. Laser scan image, *scale bar*: 10 μm. Actin fibers are stretched from one cell border to the other and form a delicate network of parallel-bundled fibers. VE-cadherin (cad-5) and β-catenin (B-CAT) colocalize at the cell border forming an interdigitated and belt-like junction zone between cells.

cadherin allows capillary tube formation and the maintenance of a single cell layered investment of vascular lumens (Matsumura *et al*, 1997; Vittet *et al*, 1997; Bach *et al*, 1998a; Halama *et al*, 1999). Saturation of VE-cadherin-dependent cell-to-cell contacts mediates growth stop at confluence (Caveda *et al*, 1996; Halama *et al*, 1999). Interestingly, VE-cadherin also allows endothelial cells to interact with fibrin and this interaction also induces the formation of capillary tubes *in vitro* (Bach *et al*, 1998a, b).

By contrast, a PECAM-1 knockout animal has no apparent vascular malformation (Duncan *et al*, 1999), indicating that functions of PECAM-1 during vessel formation can be fully compensated by other molecules, most likely by VE-cadherin itself. Moreover, PECAM-1 alone in the absence of VE-cadherin is not able to confer growth stop signals after reaching confluence and such cells do not form capillary tubes *in vitro* (Halama *et al*, 1999). The function of PECAM-1 in adherens junctions only becomes apparent when basic fibroblast growth factor (FGF)-stimulated endothelial cells are analyzed. PECAM-1 reduces FGF-induced polarization of the F-actin network towards the protruding edges of the cell and reduces FGF-induced cell scattering. Moreover, following FGF withdrawal, PECAM-1 accelerates F-actin realignment into parallel bundles and restoration of an intact monolayer.[1] These effects can be attributed to the fact that PECAM-1 reduces FGF-induced dissociation of VE-cadherin complexes and enhances their reformation after FGF withdrawal. Although the basis of this effect is still unclear, it is most likely mediated indirectly, because PECAM-1 and VE-cadherin do not physically interact. The cytoplasmic tails of PECAM-1 and VE-cadherin have no homology, thus they probably use a distinct region on β-catenin for binding.

Signals regulating the stability of the VE-cadherin/catenin complexes are only partially understood, and it appears that protein-tyrosine phosphatases such as PTPmu, PTP-1B, or LAR-PTP are involved, which associate with the VE-cadherin/β-catenin complex and thereby increase the cohesivity of the complex (Kypta *et al*, 1996; Balsamo *et al*, 1998; Brady-Kalnay *et al*, 1998; Ozawa and Kemler, 1998; Muller *et al*, 1999). Also PECAM-1 can associate with protein-tyrosine phosphatases via its src homology 2 domain binding motif (Hua *et al*, 1998; Jackson *et al*, 1997; Newton-Nash and Newman, 1999), which may be the mechanism by which PECAM-1 enhances the stability of the cadherin/catenin complex.

Taken together these data emphasize the tight regulation of the assembly of the adherens junction complex. VE-cadherin and PECAM-1 in conjunction control the maintenance of a stable vascular tree. VE-cadherin appears to be required to allow endothelial cells to respond to growth factor stimulation, as evidenced by the fact that transfectants expressing PECAM-1 only, but not VE-cadherin do not respond to growth factors (**Fig 2**). In this respect PECAM-1 appears to stabilize the junctional complex against FGF-induced dissociation, because in cells coexpressing VE-cadherin and PECAM-1, junction dissociation is limited to a short transient peak resulting in an accelerated reformation of a tight endothelial monolayer.[1]

In leukocyte transmigration The VE-cadherin knockout mouse cannot be used to assess the function of VE-cadherin during leukocyte migration, because these mice die during embryonic development; however, from several *in vitro* experiments it is well documented that the adhesiveness of the adherens junction complex controls permeability for macromolecules as well as for emigrating leukocytes (Allport *et al*, 1997, 2000; Sandig *et al*, 1997; Hordijk *et al*, 1999; Johnson-Leger *et al* 2000). It was thought that neutrophils dissociate the adherens junction complex, thereby altering the permeability of the vascular wall for permeating cells (Allport *et al*, 1997); however, other studies suggested that this phenomenon might reflect degradation by neutrophil proteases released during specimen preparation, thus being a cell culture artifact (Moll *et al*, 1998). Still the fact remained that transmigrating cells have to somehow cross the adherens junction barrier. Recent

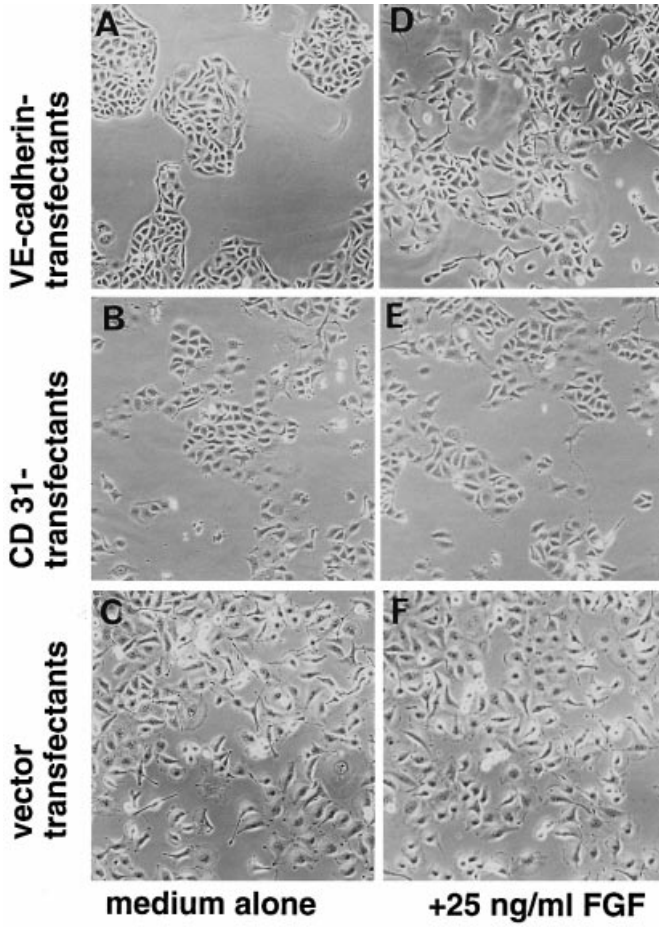

Figure 2. Transmission microscopy of ECV304 cells, stable transfected with VE-cadherin, PECAM-1. or vector alone. Halama *et al* (1999). Cells were cultured in RPMI/10% fetal calf serum (*A*, *B*, *C*) or in RPMI/10% fetal calf serum/20 ng basic FGF. Whereas in the absence of exogenous growth factors, both VE-cadherin- and PECAM-1-transfectants cluster, FGF induces cell scattering in VE-cadherin, but not in the PECAM-1 transfectants. Vector only cells scatter, whether FGF is present or not.

studies employing monocytes were able to confirm that a transient and focal disruption of the VE-cadherin complex occurs during leukocyte transmigration, which was inhibited by antibodies (Allport *et al* 2000). Moreover, studies using FGF-stimulated endothelium found that FGF significantly enhanced transmigration of mononuclear cells in dependence of the ability of FGF to dissociate the cadherin/catenin complex,[1] which confirms the concept that transmigration of cells is regulated by altering the composition of the adherens junction complex.

The situation is different with regard to PECAM-1. In the PECAM-1 knockout mouse transmigrating leukocytes were trapped within the basement membrane documenting the role of PECAM-1 during transmigration in an *in vivo* model (Duncan *et al*, 1999). Several *in vitro* experiments were able to confirm this effect of PECAM-1. PECAM-1 appears to function by providing a ''track'' for transmigrating cells between adjacent endothelial cells (Berman and Muller, 1995; Piali *et al*, 1995; Poggi *et al*, 1996; Prager *et al*, 1996; Pellegatta *et al*, 1998). Interestingly, employing transfectants expressing PECAM-1 or VE-cadherin or both molecules, the augmented transmigration of leukocytes in response to FGF seen in cells coexpressing VE-cadherin and PECAM-1 was not observed in cells expressing PECAM-1 only.[1] These experiments illustrate that PECAM-1-dependent mechanisms of directing leukocytes through junctions are controlled by VE-cadherin in a way sensitive to FGF.

In signaling It was initially thought that proteins of the adherens junction complex are cell-to-cell adhesion molecules. Subsequently, it was shown that β-catenin is not only involved in modifying cadherin-based cell adhesion, but is also able to transduce signals into the nucleus. This information mainly came from investigations of the Drosophila protein armadillo and the Xenopus β-catenin, which are both homolog to the human β-catenin. Armadillo and Xenopus β-catenin were found to be a central part of the Wnt signaling pathway. Wnt proteins bind to receptors of the Frizzled family and through several cytoplasmic relay components (recently reviewed by Behrens, 2000), the signal is transduced to β-catenin, which then enters the nucleus and forms a complex with the Lef/tcf family of transcription factors and activates transcription of Wnt target genes. A detailed description of the Wnt pathway can be found on the Wnt gene homepage HTTP://www.stanford.edu/~rnusse/wntwindow.html. This pathway has also been described to function in human cells. Currently known target genes activated by this pathway in human cells are C-myc, cyclin D, Tcf-1, matrix metalloproteinase MMP-7, peroxisomal proliferator-activated receptor (PPARdelta), urokinase-type plasminogen activator receptor (uPAR), c-jun, and fra-1 (He *et al*, 1998, 1999; Brabletz *et al*, 1999; Mann *et al*, 1999; Shtutman *et al*, 1999; Tetsu and McCormick, 1999). In primary endothelial cells, transfection with an expression vector for Wnt-1 increased the free pool of β-catenin and the transcription of a Lef/tcf dependent reporter gene construct and also induced proliferation of endothelial cells in cell culture (Wright *et al*, 1999).

The source of transactivation-competent β-catenin is still under debate: β-catenin is found in the cytosol in a complex with the protein kinase GSK-3b, the adenomatosis polyposis coli protein APC and Axin (Behrens *et al*, 1998; Behrens, 2000). It is well documented that upon Wnt signaling, β-catenin is released from this cytosolic complex and translocated into the nucleus. In addition, as described above, β-catenin is linked to the cell membrane in a complex with VE-cadherin and PECAM-1. Upon stimulation with growth factors, such as epithelial growth factor (EGF), FGF and others, this membrane-associated complex is dissociated; however, it has yet not been directly proven that membrane-derived β-catenin is indeed transactivation competent. The existence of such a second pool of transactivation-competent β-catenin is supported by the finding that the ectopic expression of high amounts of cadherins antagonizes the signaling properties of β-catenin (Fagotto *et al*, 1996; Torres *et al*, 1996; Orsulic *et al*, 1999). Moreover, in endothelial cells, FGF rapidly dissociates the VE-cadherin/β-catenin complex and translocates β-catenin into the nucleus.[1] As described above, this effect of FGF is attenuated by PECAM-1: PECAM-1 retains β-catenin within the junctional adhesion complex and reduces the FGF-induced cadherin/catenin dissociation and nuclear translocation of β-catenin to a short transient peak.[1] The functional consequences of nuclear translocation of β-catenin in the developed vascular tree are poorly characterized. By analogy to the function of β-catenin in Drosophila and Xenopus, it can be assumed that the liberation of transactivation-competent β-catenin from the adherens junction complex interferes with endothelial cell cycling, migration, and vessel formation. Thus, the adherens junction complex offers itself as a potential target for pro- and antiangiogenic treatment concepts.

CONCLUSION

The endothelial adherens junction complex controls cell morphology, organ shaping, and cell cycling. In the adult vascular tree the maintenance of a quiescent endothelial cell monolayer appears to depend on growth stop signals transmitted through VE-cadherin/β-catenin, which is further supported by the observation that mutations of β-catenin or deletion of cadherin is associated with cancer (Polakis, 1999). Under physiologic conditions, growth factors can temporarily and reversibly override this growth stop signal provided by cadherin/β-catenin in order to allow new vessel formation.

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